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An f-theta lens design for bio-medical system: Laser scanning microarray reader

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Abstract. Laser scanning technology has put in microarray image system for its characteristic of non-contact measurement, high performance, and good sensitivities for the development of bio-technology. In the requirement of metrology, microarray biochip image has become more precisely and non-contact measurement like optical detector or ultra wave sensor is plenty applied in the imaging system. The paper provides the new type construction of laser con-focal microarray scanning and fluorescent detection system. The enabling key component, f-theta lenses, is designed as micro-meter focusing scanning lens. The scanner exploits the functional advantages and the optical system has small spot size, great linearity and large depth of focus. The system is expected to be simply operate, small hardware size, and fast speed. The optical design of the scanning lenses and the fabrication of scanner are also introduced and discussed.

Key words: biochip image, f-theta lenses, laser con-focal microarray scanning

1. Introduction

Bio-technology is one of the most potential industries in 21st century, where the growth of biochip market is highly expected. Among the various applications, DNA Microarray System, which can produce massive amounts of data and analyze the image simultaneously, have already been viewed as the most powerful type of biochip. Due to the industry survey of Business Comm., the worldwide market of microarrays is about US$226 million in 2000, and would be increased to US$536 million in 2005, with 18.9% growth rate per year. (http://www.bccresearch.com/editors/RB-148.html) The key points of market growth is depending on the manufacture cost of biochip, selling price, the feasibility of enabling technology, and the purchase by research institutes and clinical diagnosis.

Microarray scanner, containing the image scanner and analyzer software, plays a more critical role in the whole Microarray System. According to the same report of Business Comm., the worldwide market of microarray fluorescence scanner is about US$86 million in 2000, and would be increased to

US$224 million in 2005, with 21.1% growth rate per year. (http://www.bcc-research.com/editors/RB-148.html) The basic functions of microarray fluorescent scanner are to excite the fluorescent dyes on the microarray chips and to collect emitted light to form the image. Due to necessarily high resolution to guarantee its accuracy, image scanning technology, traditionally appeared in projectors or laser printers, has been applied to this new field. The scanning technique is not only can be utilized to record data on a medium, such as machining, welding, printing and projection; but also can be put in medical use instruments for gene recognition, while an enough resolution it can provide. Because of the improvement of optoelectronic technology and semiconductor manufacturing process, it is possible to produce this kind of scanner with high sensitivity and low cost.

Considering the fluorescence scanner for imaging of DNA Microarray, there are two main approach platforms in the industry. One is composed of laser light source and photomultiplier tube (PMT), which is implemented by PerkinElmer, Axon, Genomic Solution, BioRad, etc., and the other is composed of white light source and charged-coupled device (CCD) camera, which is chosen by Alpha Innotech, Applied Precision, etc. These two approach platforms have different advantages respectively, but the former platform, which has laser sources and PMT, is more popular due to its lower cost about US$50,000–80,000. The research is based on the development and improvement of the first type platform.

There are traditionally three kinds of mechanisms to achieve two dimensional microarray scanning in this platform. First classification is the loader and light source with single-axis stage respectively. Second one is the loader with dual-axis stage and fixed light source. The third one is the light source with dual-axis moving. The unique improvement in this paper is using the combination of scanning lenses and a periodical moving mechanism replaces the expensive stages. In the following paragraph, the basic structure of the presented example includes sample loader with single-axis moving stage, one fixed light sources, con-focal fluorescence detection, and especially the f-theta lens set.

2. System requirements

For the study of entire genomes expression or large sets of genes, dot–blot hybridizations, which the gene-specific nucleotide sequences of probes are bound to membranes in microarray, would complete a fast and precise examination. As mentioned above, one way to detect dot–blot hybridizations is to get the fluorescent images by laser scanning mechanism that has one or more wavelength laser light sources, and to compare the fluorescent signal with the known control probes and RNAs or cDNAs (Kain 1998).

Before paying attention to the f-theta lens, the environment parameter designed in this study should have a clear description to define the specification of the lens design. The example system setup is shown as below (Fig. 1). The laser beam goes through mirrors, dichroic beam splitter (1) and (2) toward to the polygon module and then passes through the f-theta lens set spotting on the sample. The spotting light will stimulates the fluorescence dye and the stimulated fluorescent light would trace the same optical path back to the beam splitter (2) along with the reflected light and then would pass through the filter. The filter will block the stray light but let the stimulated light go through and be detected by the PMT module. The detected signals will then be processed by the bio-information systems (Tomei *et al*. 1988).

In this system, two popular materials, Cyanine3 (Cy3) and Cyanine5 (Cy5), are applied as the fluorescent dye samples as their excitation and radiation characteristics are shown in Table 1. The selected dichroic beamsplitter and optical filter can separate light with different wavelengths of excitation light and radiation light of the fluorescence dyes. These two pass bands allow the fluorescent radiation of the two samples passing through the beamsplitter so that the desired intensity distribution of radiation light would be obtained.

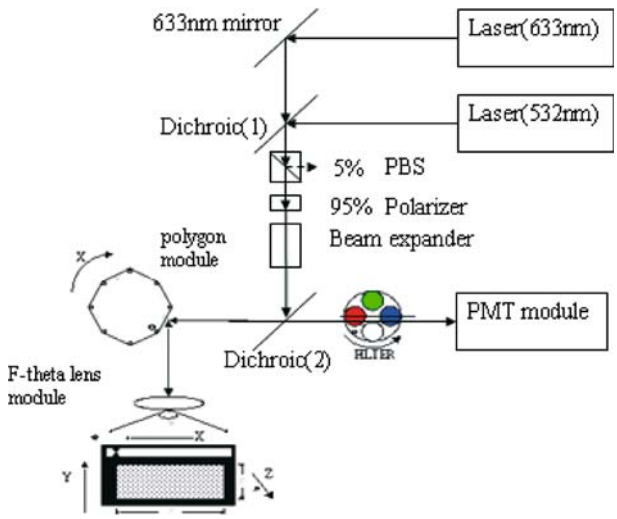

Fig. 1. Example system setup.

Table 1. Excitation and radiation characteristics of Cy3 and Cy5

	Excitation (nm)	Radiation (nm)
Cy3	554	568
Cy5	649	666

To find a suitable detector with good performance, photomultiplier tube (PMT) and Avalanche Photodiode (APD) are both good candidates, which the comparison table between them is shown in (Table 2). The receiving speed of PMT is about 200 KHz, the cost range is from NT$40 000 to 50 000, and the sensitivity gain is from 1 to 100 000. On the other hand, the receiving speed of APD could go up to 2 GHz, the cost range is from NT 10 000 to 100 000, but the sensitivity gain is from 1 to 100. Because weak fluorescent response needs high sensitivity detective sensor, PMT is the better choice in this system.

Considering the scanning geometries using rotary scanners, which are possibly manufactured in local supplier, rotating polygon and vibration mirrors are two selective items, listed in Table 3. Vibration mirror is easily to be made for local manufacturers in Taiwan but its scan speed is fatally limited below 800–1000 rpm. Its another strength is competitively lower cost than high precision polygon mirror. However, scan uniformity, reliability, and life time of vibration mirror need to be especially concerned. On the other hands, the rotation speed of Polygon mirror can easily meet design specification to fulfill the read speed of microarrays. There are some manufacturing tolerance problems in high precision polygon mirror but it is still better for the current needs of high speed scanning function in microarray application.

Usually, the individual probe molecules on dot–blot microarray are well arranged orderly on a 22 mm × 75 mm substrate with 80–250 dpi. That means the diameter of sample spot is about 25–500 μm, generally 100 ± 50 μm. To calculate the fluorescent intensity of one spot precisely, the spot needs to be divided to many tiny pixels and summed up the intensity of all pixels. When the target is under-sampled by the pixels, the images are barely detected. If increasing the density of the pixels such that they overlap one another, a clearer image will be detected. Thus, the scanner should

Table 2. Comparison between PMT and APD

Solution	Scan speed	Cost	Application case
PMT	Up to 200 KHz	NT$40 000–50 000	High sensitivity image sensor
APD	Up to 2 GHz	NT$10 000–100 000	High speed image sensor

Table 3. Comparison between polygon and vibration mirror

Solution	Scan speed	Cost	Application case
Polygon Mirror Module	possibly up to 4 500 rpm	High precision: NT$200 000 Mid-end (20 μm): NT$10 000	Off-axis rotation scanning
Vibration Mirror Module	only up to 800 rpm	Mid-end: NT$10 000	Low speed scanning

provide higher resolution than the size of the element on the microarray. For example, the suggestion pixel size is 5–20 μm for a microarray with 100 μm diameter spot. It means the pixel resolution is 5000 dpi or the better spot size has to be less than 5 μm.

Besides, other considerations include scanning along the 75 mm width, 5 cm diameter of circumscribed circle of polygon, dual laser sources (635 nm and 532 nm wavelengths) and 10 cm $\times$ 10 cm $\times$ 20 cm opto-mechanism size. The numerical aperture is around 0.75, and scanning speed can reach 20 lines per second. The superiorly ability of con-focal microarray scanner is expressed on off axis, high speed, low cost, high sensitivity, and sequential image.

In such a scanning optical system, when a polygon mirror is used for a reflector, an inclination error of the reflection surface of the polygon mirror often causes dislocation of one-line images that are formed on the scanning surface, i.e. uneven differences along the one-line scanning path. Moreover, the requirement of pixel size and linearity in the optical system applied in microarray scanning is close to micrometer scale, that is thousands times actually than the scanning system applied in laser printer that invented in 1970s.

The goal of Microarray Scanner Systems is precise position, fast scanning, high sensitivity and resolution, and the proper dying for most visible light. For this requisite, this paper uses the famous optical design software, CODE V, which results are verified by millions of practically existing use worldwide, to design a new optical system with polygon mirror to achieve the needs.

3. *f*-Theta lens design

To achieve the system requirements, the most important key component of this system is the optical scanning lens, *f*-theta lens set. The type of the scanning geometry in this research is so called one-dimensional pre-objective scanning using simple rotating polygon for reflecting (Kain *et al.* 1996). In pre-objective scanning, the focus lens diameter have to be greater than the beam diameter and well adjusted for off axis images so as to produce a flat field on the target. The scan distance, or image height, is corrected to a linear function of the scanning angle by the *f*-theta lens. Also, the twelve mirror facets of a symmetrical polyhedron provide additional sequential reflecting surfaces (Iizuka 1998). The sweep angle of the lens is set to be 30 degrees, though the maximum angle of this polygon is up to 60 degrees due to the cost down of polygon manufacturing. Additionally, the other key function of this lens set, mentioned in system requirements, is to concentrate a deflected beam on the scanning plane with 5 μm

spot size and to make the image height is proportional to the scanning angle with a linear scan velocity. For the majority of excitation light source for microarray, the incident wavelength ranges from 630 nm to 530 nm.

Considering the axis of polygon rotation, it is displaced by a distance from the plane of the facet mirror but is still parallel to surface. The apparent center of scan of the reflected beam is no longer a stationary point although the reflected beam sweeps at the double angular rate of the mirror, just shown as below (Fig. 2). It is a locus leading to the offset of the image height, which interference the linear scanning velocity. The f-theta lens must compensate for it to guarantee the request specification (Minakuchi 2000).

All the individual lens in the set are spherical lens without exception in order to make sure the lowest manufacturing cost. The aspherical lens has better performance in the same material and the similar dimension but the die cost, assembly issues, and the testing modules will push the design use the spherical one, especially the end product amount is not very large.

Because the scan target is a 3 inch width microarray chip and calibration fragments are necessary in the head and tail of scan line, the scanning field needs to be a little more than 3 inch. Besides, it should be noted that the number of spots in this system is not defined by the scan equation as below (Ormend 1994):

$$N = \frac{\phi D}{a\lambda}, \tag{1}$$

where N is the number of spots, ϕ is total sweep angle, D is the width, or aperture, of the scanner polyhedron, a is the aperture shape factor, and

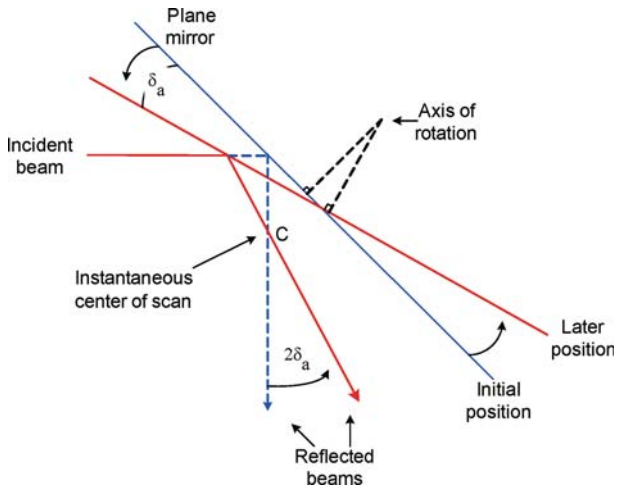

Fig. 2. Diagram of the axis of polygon rotation.

λ is the wavelength of laser source (O'shea 1985). In tradition, the scan equation determines the spots at most applications. However, the spot number in the system of this study is decided by the received signal processing speed of photomultiplier tube. In this case, the limitation is 200 kHz so that the spot number is 15 000, smaller than which obtained from the equation.

Under all these conditions, the prototype design, selecting the starting point from Milton Laikin's three-lens design (Laikin 1991), is shown as the Table 4 and drawn as the figure in central and oblique incidence (Fig. 3). This four lenses spherical solution takes advantages of popular materials and is feasible in practical manufacturing. The only weakness of this design is the curvature radii of the first lens is a little close. Generally speaking, the radii of this lens may as well have the constraint as below:

$$\frac{12.1}{R1}+\frac{14.1}{R2}>0.32 \quad \text{or} \quad \frac{12.1}{R1}+\frac{14.1}{R2}<-0.32. \tag{2}$$

Even though the radii are close, the centering still can be solved by better tools.

In order to show the performance of this lens set, the scanning angle of polygon is divided into 15 000 parts to fulfill the spot number specification mentioned above. The most severe conditions in linearity and spot size all happens in the edge. For convenient and pithy representation without loss of generality, the scanning angle is divided equally into one-tenth to indicate 11 positions scanning from edge to another edge along the diameter of lenses. Here, only six representative positions (clarified as the following illustration) are marked due to symmetry (Fig. 4). Obviously, the result perfectly achieves the two major specifications in spot size and linearity. The spot sizes at the six positions are all in the safe range, or 5 μm (Table 5). Further, there are 1 500 spots between any two positions so the five differences of scanning distance mean that the scanning length between two spots are below 0.002 μm. It would be noted that scanning linearity is proven due to the immeasurable error.

Table 4. The lens prototype design

	Radius (mm)	Thickness/distance (mm)	Semi-apeture (mm)	Material
G1R1	6–9	2.59	5.70	SLAM2_OHARA
G1R2	9–13	0.30	7.10	air
G2R1	105–125	4.00	8.85	SBSM15_OHARA
G2R2	17–23	9.56	9.50	air
G3R1	15–21	5.25	11.35	STIH13_OHARA
G3R2	55–75	3.24	14.50	air
G4R1	110–135	6.40	17.00	SBSM4_OHARA
G4R2	23–36	74.71	40.00	air

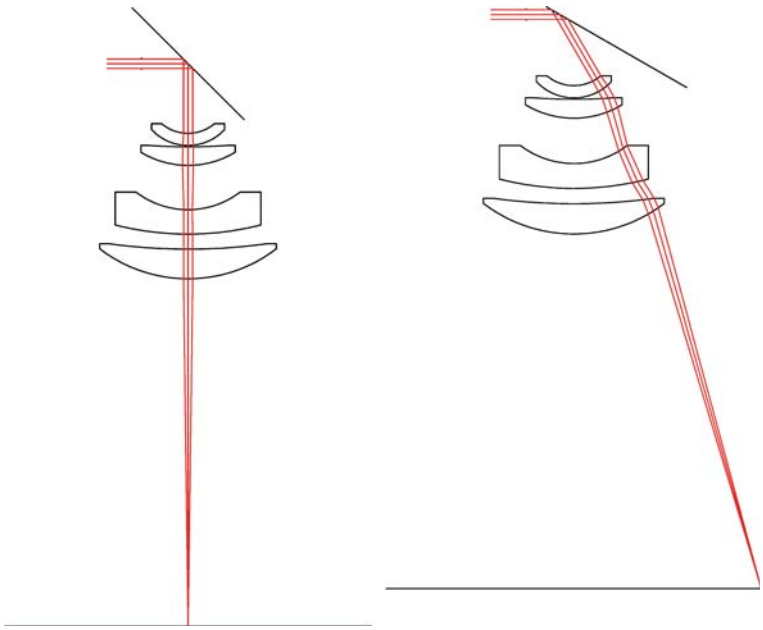

Fig. 3. Drawing of the f-theta lens set.

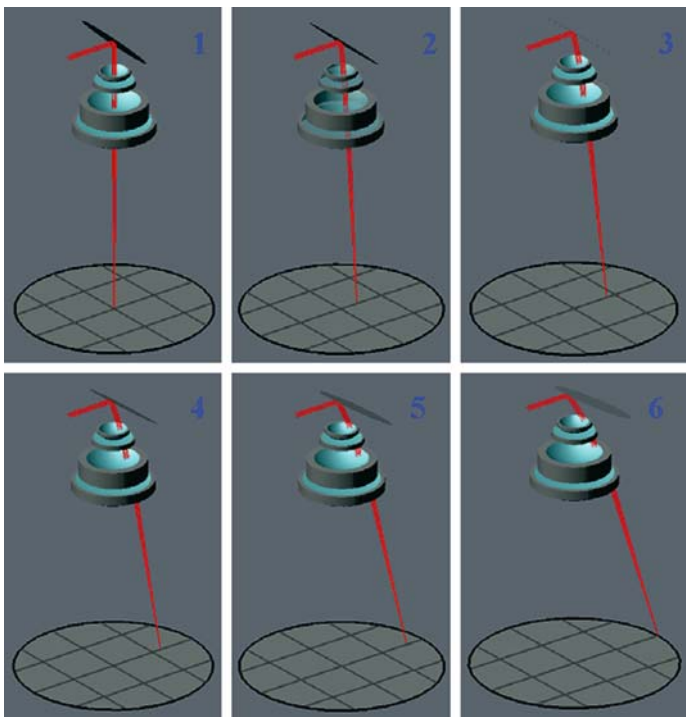

Fig. 4. Six representative positions of scanning.

Table 5. The spot sizes at the six positions

	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
Scanning angel	0°(central incidence)	3°	6°	9°	12°	15°
Spot size (μm)	0.25745	0.32293	0.47502	0.63350	0.79760	2.3852
Scanning distance (mm)	–	7.8698	7.8777	7.8885	7.8908	7.8663 –

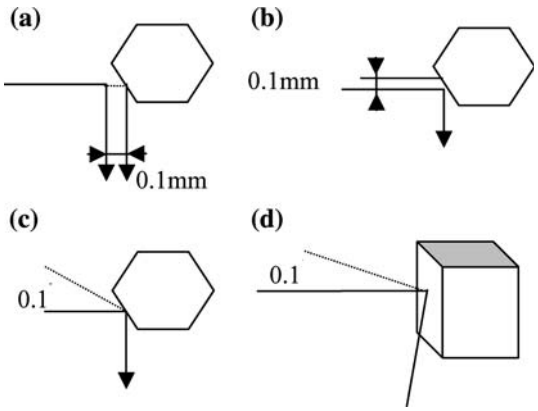

Fig. 5. Diagram of the system alignment error.

4. Tolerance analysis

The manufacturing errors basically come from lens distance, lens thickness, decenter difference, lens tilt angle, and system alignment. The error amplitudes listed as below are treated in this tolerance analysis and the system alignment error is regarded as the Fig. 5. The tolerance criterions include two points: (1) spots size along the scan line is below 5 μm, and (2) the scanning velocity keeps linear, that is, the scanning distance of the six representative positions is allowed to have 0.1 mm error compared with the original design result (Table 6).

Table 6. The error amplitudes in the tolerance analysis

		NR	IRR	Surface optical axis tilt (arc min)	Element optical axis tilt (arc min)	Lens central thickness (mm)	Outer aperture (mm)	Lens distance (mm)
G1	R1	3	1	1	1	± 0.03	$-0.005/-0.02$	–
	R2	3	1	1				± 0.01
G2	R1	3	1	1	1	± 0.03	$-0.005/-0.02$	
	R2	3	1	1				± 0.01
G3	R1	5	2	1	1.5	± 0.03	$-0.005/-0.02$	
	R2	5	2	1				± 0.01
G4	R1	5	2	1	1	± 0.03	$-0.005/-0.02$	
	R2	5	2	1				–

The analysis shows the most errors are much below tolerance and the linear velocity requirement is all passed in all conditions for G1–G4 lens. However, the element title angle makes G2 and G3 have a 6 μm spot on the position 6 and the lens thickness makes G1 have 10–15 μm spot size. For the original design specification, the sorting of finished lens set is necessary. Considering the rougher resolution is another market segment, the inferior finished goods can be used on 20 μm spot size scanning system. All things in their being are good for something.

5. Conclusions

This paper has presented an optical scanning system which exploits the functional advantages and the optical system has small spot size, great linearity and large depth of focus. The f-theta scanning lens has been successfully developed, which use spherical lens to be focal lens. All the lens materials in the image forming optical system are easy to find, and the scanning optical system satisfies the conditions bio-scanner needs. In a scanning optical system where a bundle of rays scans a surface to be scanned through an polygon mirror adding on a servo motor and an image forming optical system, the image forming optical system includes a positive front lens group having two lens elements and a rear lens group constituted by an elongated single anamorphic lens element having a strong positive refractive power in the sub scanning direction. Most importantly, the feasibility is verified by the simulation and tolerance analysis successfully.

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